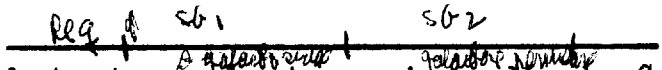
Lecture 3, Columbia University. April 13, 1964

Title · Part I 1.) A. Bacteria:

I. Gene control mechanisms in Higher Organisms; Two common types and several special types.

1. The inducible systems: Enzyme needed to break-down a product from the environment: some carbohydrate, as example. Example, galactose.

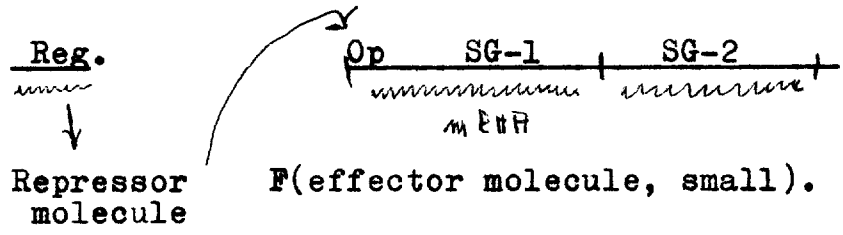
a). The Lac. locus: 

b). Gene active when substrate, galactose, in medium. Gene very low in activity when no substrate. Substrate contols degree of activity of the gene.

c). This substrate, called the inducer; induces formation of the enzyme.

d). Related substances act as inducers; enzyme, beta-galactosidase can not act on these related substances. These useful in study of enzyme formation and thus, rate of gene action with substrate.

2. The Model for control of gene action: Regulator and Operator relationship:

a). Inducible system: 

R + F ⇌ RF . Substrate -galactose: Attaches to R. R kept from sitting on operator region and blocking gene action.

Substrate, small molecule, low or absent; R is not bound. Sits on operator region of gene: no gene transpcription.

3. The "repressible" systems: Genes in biosynthetic pathway. End product controls the degree of action of the genes:

Same model: Now, R and F(small molecule) when combined, sit on operator region of first enzyme: no gene transcription. When F substance limited, R is freed of F; this results in unbinding with the segment, operator, at beginning of operon.

4. The mRNA in bacteria: examined cases, short lived; functions several times and is distroyed. Must keep making mRNA.

In higher organisms, some mRNA, such as that for haemoblogin, long-lived.

Mechanism of breakdown or protection from breakdown not known.

Advantage to bacteria of breakdown of mRNA and replacements of mRNA. Constant change in environment; constant change in life cycle when food readily available. Requires changes in action of different genes to accommodate this.

5. Detection of the regulator genes: Through mutations of several types: Gene action no longer controlled by substrate: Locus of change at region not in structural gene. Other mutations: block transcription altogether: site of mutation at same locus.

6. Detection of changes in operator region: Gene transcribes all the time, regardless of substrate concentrations: Locate site of mutation at end of first gene in operon.

   Genes not transcribing at all: Site of mutation: at beginning of the operon;, i.e., at same position.

7. Studies of inducible and repressible enzyme-gene relationships: show that cytoplasmic constitution and gene action intimately related. A cycle of events from cytoplasm to gene and to cytoplasm. This type of gene control very prominent in all organisms. Substances in cytoplasmcontrolling specific genes very different, in many cases, than substrate inducers or end-product of bio-synthetic pathway controls. Will consider.

8. A Special Type of Control of gene action in Bacteria: Not cytoplasmicall controlled. Reflects a strictly genetic control system.

   Example: The duplicate genes for flagella proteins: flagella antigens:

   Mutant sites: antigen type.
   Only one gene active at any one time; other gene repressed.

   Control of which gene active, resides in the Regulator:

   Regulator undergoes changes in phase of activity: active - inactive- active.

   When Regulator is active: H_2 gene active. When Regulator gene is inactive; H-2 gene repressed, H-1 gene activated.

   Duration of cycle of activity of the regulator: Surprising results:

   Long duration of inactive phase: Monophase-1. Change to active phase in single bacterium: its progeny now show change from action of H-1 gene to action of H-2 gene; H-1 gene repressed

   Long duration of active phase: Monophase-2. Changes, same as above

   Bacterium: undergoes frequent chantes in phase in progeny: Diphase.

   Duration of phases: quite different after isolation following a particular phase.

   Control of duration of phase: locked into the regulator: The regulator is a GENETIC CLOCK . GENETIC TIMER.

II. Examples of control of duplicate genes or of alleles, alternately, in higher organisms.

A. Lactic -dehydrogenase

1. Lactic -dehydrogenase enzyme: a muscle enzyme. In mature individual, this enzyme differs in muscles: Skeletal muscles, quick acting, have M enzyme, produced by one gene. Heart, rythmic, slower action, have H enzyme, produced by another gene.

2. Embryos in organisms with both types of muscles: Enzyme in early development from H gene only; M gene repressed.

3. Transition period in skeletal muscles, both ~~type~~ products present in cell - H gene product and M gene product. In heart, only H type as M gene permanently ~~p~~repressed.

4. The lactic-dehydrogenase: composed of 4 sub-units.
   In Heart: H_4; In mature skeletal muscles: M_4
   In transition tissues - skeletal muscle- find three other types: Composition: H_3M_1; H_2M_2, H_1M_3. This shows relationship of Hgene and M gene subunits.

5. Physical and chemical properties of H_4 and M_4, nevertheless, not the same.

6. Modifications of control of these genes by addition of hormone: Estradiol. When introduced into uterus of female with young embryos, embryos develop some M subunits: activation of M gene. When hormone removed, return to production of H sub-units

   Estradiol: to chicken heart cells in culture: Force them to produce some M sub-units. When removed, this gene repressed and only H subunits produced.

7. Relation of muscular-dystrophy to action of H and M genes: Change to M gene action disturbed; ineffective M gene product or not enough. Either gene change or control mechanism change.

III. The place of action of hormones:

1. Hormones, known to activate certain gene products specifically in only certain cells.

2. Discovery of relation of hormones to activation of specific genes: Discovered in Chironomous: ecdysone, activating genes related to pupation process: (Has been discussed here earlier.)

3. The place where the hormone acts: First evidence very recent: This shows: hormone goes into the nucleus. In one case tested, hormone goes into nucleus or resides at periphery of nucleus:

4. The test: Toad bladder epithelium: controls passage of speciric substances, one of them Na.
   a). Discovered: control of Na-pump, that is, control of passage of sodium is by ephthelium cells of bladder.
   b). Can be made to start this by adding hormone Aldersterone
   c). Test of where hormone goes when added to toad bladder cells:
      (1) Hormone made radioactive.
      (2) Cells autoradiographed: Found hormone entered only the epithelium cells; only in nucleus; and at periphery of nucleus: Slide 1.

5. Reason why hormones now being studied activily for relation to activation of gene: relation in structure to bases in DNA. Expect very great increase in knowledge of this control very soon.

IV. Mechanisms of control of gene action in differentiation; continued.

A. Mosaic Cytoplasms.

1. Control of gene action by differentiation of the cytoplasm into regions, each with different composition: At varies different times in development, this must occur.

2. Example: the mosaic egg cytoplasms: used in last part of last centruy and early part of this century in "hay-day" of embryology.

(a) Nucleus of egg, before meiosis: manufactures many different substances; places them in specific parts of the cytoplasm.

(b) Some eggs: these different substances very conspicuous, visually. Egg is visually a mosaic.

(c) Study of development: could relate different parts of egg to development of different parts of organism after cleavage divisions following fertilization.

3. Visible mosaic cytoplasm at later stages in development: Development of stomata in leaves of plants:

(a) Function of stomata: to control intake of gases and loss of water when water is low, or transpiration high.

(b). Function controlled by two cells: Diagram: Open and close an air space by their movement:

(c) Development of stomata: Appearance of stomata in maize leaf: Slide 2 Found in rows: parachemya cell between each.

(d) Rows of cells: laid down very early in embryo development: Note pattern of laying down of cells.
The development of the stomata and the subsidiary cells: Slide 3. Diagram of events: Slide 4

(1). The cytoplasmic differentiation of each cell in row:

(2). The guard-mother cells formed after division: and the parenchyma.

(3) Formation of the two guard cells

(4) Formation of the subsidiary cells: Induction from the G.M.C. Differences in cytoplasm and nuclei after division.

(5). Abnormal types of events: part of study of causes: Slide 5.

4.

4. The lessons from study of development of stomata:

(1). Pattern: cells in embryo: certain ones selected in precise pattern to form row of cells with quart-cell future.

(2). Visible mosaic cytoplasm before division of cells in this row: Unequal deivison of cytoplasm: two different cells as consequenc
Mosaic cytoplasm during development. Unequal divisions of cytoplasm

(3) Induction of cytoplasmic change from GMC to adjacent cell in adjacent row. Control of division plane; result of mosaic cytoplasm. Induction: like hormone control.

V. The Built-In Genetic Timers in higher organisms.

1. This clearly shown in gene control in Paramoecium and Tetrahymena.

Genes for: Mating type protein; cilia protein - antigens; esterases; phosphotases.

2. The life cycle of Paramoecium: Diagram:

3. Micronucleus: the germ line; Macronucleus: the soma; dies. (Dissolved during meiotic process.

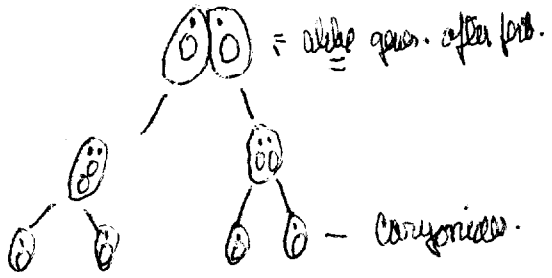
3. P. aurelia: 12 known genes for cilia protein type. Only one gene active at a time; all others repressed.
Environmental alterations - temperature - can change from one gene to another in action: active gene repressed, another gene activated; all other genes remain repressed.

4. Tetrahymena - mating type protein: 7 mating types; only one active in heterozygous individuals. Only one locus for 7 mating types.

a). The mating type process: Slide 6

b). Process: from adhesions of special cilia membrans: Slide 7.

c). Time of setting of which mating type will be expressed: In fourth cell after meiosis and fertilization: Diagram.



d). After setting occurs, no known means of altering this setting in the Tetrahymena studied, while organisms reproducing by fission.

e). After meiosis and fertilization: process begins all over again. Macronuclei discarded; resetting from genes in the micronucleus- unmodified by macronuclear type in parents undergoing conjugation and fertilization.

5. Above: Examples of control of action of only one of several different genes for same end producte; and only one of two alleles in same nucleus.

6. The control mechanisms: Can compare with the setting of the X chromosom in mammals: In Females XX : One chromosome(or part of chromosome) inactivated by structural process: Timing early, probably; Which chromosome - varies frm in different cells at time of setting:

$\underline{X}^1 X^2$ $X^1 \underline{X}^2$ In same organism: Mary Lyon, mouse: Slide 9
Photo, early development, rabbit Slide 8

Only one X active when more than two present:

XX XXX XXXX XXXXX : Like repression of all but one gene, when duplicates present; Suppression of one allele, similar.

Conclude: Behavior of X chromosome control mechanism and that in Paramoecium and tetrahymena: Now, not so strange to consider mechanisms of this type.

7. Control mechanism and phase variation in Paramoecium:

a). B. multimicronucleatum: Diurnal change in mating type, without fission of organism.

Gene found: Sets mating type rigidly, as described above. Blocks diurnal phase variation.

b). Must consider control mechanisms that could do this: maize types have some aspects related to all of this.

VI. Controlling elements:

1. Controlling elements in X chromosome of Sciara: Reviewed.

a). Emphasis, at this point, on setting of the controlling element: Germ line of male:

Resetting in germ line of female.

b). Controlling element exists in at least two alternating "states".

2. Other examples of control systems affecting chromosome behavior: The B-type chromosomes in plants.

a). B-chromosomes: not part of normal chromosome complement; No known gene action produced by them. Origins unknown.

b). The piling-up of B-type chromosomes: Maize - 30 and effect.

Grasses

c).The appearance of the B-type chromosome in several types of plants: Slides 10 - 15.

3. Behavior of B-type chromosomes in microspore-pollen development:

a). Diagram

b). Control of this: Maize: B-autosome translocations: Diagrams:

c). Control of non-disjunction: requires distal part. How shown.

d). Similar control in Rye, Festuca, Briza, Holcus, Phlem: when centromere fragments present: some require distal part for non-disjunction control: Diagram.

g). Must assume that a controling element - a regulator is present in one position. Not yet identified as with Sciara.

V. Regulator genes in higher organisms: The pattern controlling regulator genes.

1. Examples: Distribution of pigment in flowers: Same pigment in different parts of flower; or different pigments, but each shows a particular pattern of distribution.

   Distribution of one pigment in plants: Anthocyanin - red - pigment. Present in certain parts, not present in others. Intensity differs. Alleles that control both distribution and intensity.

2. Control of end product of gene action - is distribution in developmental process. Not regulated by cell contents. Like setting genes in paramoecium.

3. Excellent example of pattern regulator: Elytra of lady beetle: Pigment patterns: black on yellow. Series of alleles at one locus. Both alleles operate when together. Each allele determines particular pattern. Slide. 96

Behaves as if Regulator at gene: *** Reg.

This may not be so: maize cases: One regulator; differences in pattern due to state of operator at gene locus: Slides 17 to 18

A gene; each with operator in different state.
One regulator controlling pattern of action of this gene.

VI. Origin of studies of control systems in maize.

1. Initial experiment: chromosome type of b.f.b. cycle.
2. Expected results -
3. How experiment conducted.
4. The b.f.b. cycle seedlings: self of recovered branches.
5. The seedlings: Many selfed ears gave altered gene action; unstable.
6. The transplanted seedlings: plants; chlorophyll types. Changes in rate of gene action: Slides. 19 to 22
7. Conclusions: controlling elements responsible for altered gene action.
8. Types of change in chromosomes of complement as result of b.f.b. cycle.

Slides - Lecture 2

1. Toad bladder cells - autoradiograph of bladder cells with hormone, aldosterone
2. Stomata - maize leaf -
3. " devel of mut - Stebbins.
4. " " " - Stebbins - early stage Row of GMC + parenchyma cells.
5. " abnormal types - Stebbins -
6. Mating in Paramecium - Sonneborn
7. Cilia membrane - responsible for mating act -
8. ~~mouse~~ Cell, early dev. rabbit - x ch. at membrane.
9. Mouse - coat - Mary Lyon
10 B-chr - Festuca arundinacea
11 " " " " (fig 8 9). F. pratensis, fig. 10, 11
12 " " Holcus
13 " " Phleum nodosum
14 " " Phleum nodosum
15 " " maize
16 - Elytra, Lady Beetle.
17 - Ear maize - 7522 (9) x 7708 A (3)
18 " " 7896 B (3) II x 7912 (8)
19 " " of 17 - enlarged
20 " " of 18 "
21 - 25. Leaves of maize var. lu

Paramecium + Tetrahymena:

1) Surface proteins and genes - mating type; cilia antigens.

2. P. aurelia - (1) 12 or more different, non-linked genes, for cilia antigens -
(2) Only 1 gene active at a time.
(3) Can induce change (temperature) in antigen type - this gene turned off and another gene turned on.

3) Some Tetrahymena: Two alleles present in cell nucleus. About 4 divisions after zygote formation, one allele is activated, other allele turned-off. Permanent setting until next meiosis + zygote formation

4). P. aurelia - 2 mating types -
T. pyriformis - one locus - potential for several mating types
(1). Shortly after zygote formation, one mating type gene activates one. Rigid setting. No change until another meiosis + zygote formation. Permanent inheritance of cell clone:
(2). This resembles permanent inactivation of X chrom in mammals:
Tabby mouse ♀: { X X set or X X } X XX set X XXX set

5) P. multimicronucleatum:
(1) mating type - diurnal change without fission.
(2) "gene" (regulator) found: Blocks diurnal change and induces permanent, rigid setting of action of one gene.